Animal Health Research Journal Vol. 6, No. 1, March 2018 pp. 98-107

ISSN: 2356-7767

Disposition kinetic of clarithromycin in healthy and *Escherichia coli*- infected broiler chickens

Hesham, S. Taha and Riham, M. Raafat

Biochemistry, Toxicology and Feed Deficiency Dept. (Pharmacology Unit), Animal Health Research Institute, Dokki, Giza.

Received in 16/1/2018
Accepted in 19/2/2018

Abstract

The pharmacokinetics of clarithromycin were studied in both healthy and *Escherichia coli*-infected broiler chickens following intravenous (I.V.) and intramuscular (I.M.) administration at a single dose of 10 mg/kg b.wt. The pharmacokinetic parameters were measured in serum samples by microbiological assay using *Bacillus subtilis* ATCC 6633 as tested organism. After I.V. and I.M. administration in healthy and infected broiler chickens, the disposition kinetics of clarithromycin were described by a two compartment open model .

In infected broiler chickens, a significantly decrease in the maximum serum concentration (C_{max}=1.66 ± 0.02 ug/ml), lower volume of distribution (V/F : 3.26 ± 0.08 (mg) / (µg/ml)) and non -significant decrease in elimination half-life ($t_{0.5(\beta)}$: 6.04 ± 0.20 h) and mean residence time (MRT : 8.4 ± 0.28 h) were found after I.M. administration

Furthermore, the infection status reduced absorption of I.M. administered clarithromycin, significantly decreased area under the curve AUC_{0-t} (13.53 ± 0.14 µg/ml*h) and the maximum serum concentration but increased T_{max} (1.09 ± 0.03 h).

The T>MIC values (12 h) of clarithromycin suggested that the drug is clinically effective in the treatment of various infections caused by E.coli (strain O_{76}) by intramuscular administration. In conclusion, to maintain a minimum therapeutic concentration of clarithromycin, a satisfactory dosage regimen of drug would be 10 mg/kg intramuscularly repeated at 12 h intervals in infected chickens.

Keywords: *Clarithromycin, Escherichia coli, broiler chickens.*

Introduction

Large amount of antibiotics used for human therapy, as well as for farm animals and even for fish in aquaculture, resulted in the selection of pathogenic bacteria resistant to multiple drugs. Bacterial infections may be life threatening or cause great economic losses in human being and animals, so antibacterial intervention is therefore a critical issue. However, developing resistant bacterial strains remain a constant medical problem due to the frequent use of classical antibiotics. Introducing new antibacterial agents may solve such problem. **(Hiroshi, 2009).**

Clarithromycin is a macrolide antimicrobial agent which achieves considerably greater concentrations in pulmonary epithelial lining fluid and alveolar macrophages than either erythromycin or azithromycin **(Conte *et al.*, 1995 and Patel *et al*., 1996).**

Both Azithromycin and Erythromycin are macrolides. Azithromycin (10 mg/kg every 24h) and clarithromycin (7.5 mg/kg every 12h) have been proposed as alternatives to erythromycin for the treatment of infections with *Rhodococcus equi* in foals. Compared to erythromycin, these drugs are more chemically stable and achieve higher concentrations in phagocytic cells and tissues. **(Steeve *et al.*,**

2004).

Activity of clarithromycin and its metabolite,14-hydroxyclarithromycin in combination in vivo , was synergistic or additive for *Haemophilus influenza* **(Hardy *et al.*, 1990) .**

Also clarithromycin was active against both penicillin sensitive and penicillin resistant *Streptococcus pneumoniae* and was as active as ciprofloxacin against *Branhamella catarrhalis* **(Bryan *et al.,* 1990) .**

Their spectrum of activity includes mostly Gram-positive microorganisms, most *Mycoplasma* spp., some *Chalmydiae* as well as some Gram-negative pathogens such as *Haemophilus influenzae*, *Campylobacter jejuni*, *Bordetella* spp. and *Mannheimia haemolytica* **(Alvarez and Enzler , 1999).**

Bioavailability after oral administration of clarithromycin (7.5 mg/Kg b.w.) to broiler chickens was found about 66% and adequate drug concentration was recorded up to 12 h post administration with plasma protein binding capacity of 52%. (**Hanady *et al.,* 2016)**.

A number of studies have shown that the pharmacokinetics of drugs can be influenced by the pathophysiological changes during an infection **(Baggot, 1980**), so it is important to evaluate the pharmacokinetics of the drug in infected animals as well as in healthy animals. The plasma concentrations of cefquinome following repeated intramuscular administration of 2 mg/kg (bw) once daily for three consecutive days in normal and experimentally *Salmonella entretidis* infected chickens showed lower significant values recorded in experimentally Salmonella entretidis infected chickens than in normal ones. (**Mossad *et al.,* 2015a**).

However no information is available about the pharmacokinetics of clarithromycin in infected broiler chickens at dose of 10mg/kg bwt either in healthy and *Escherichia coli*- infected broiler, so the aims of the study were to investigate the disposition kinetic of clarithromycin in healthy and *Escherichia coli*- infected broiler chickens after intravenous and intramuscular administration .

Materials and Methods

Drug: (Klacid)^{R}, Clarithromycin, supplied as a lyophilized form in a 10-mL vial equivalent to 500mg of Clarithromycin for i.v. administration., the manufacturer is Abbot/France. Reconstitution according to label directions, resulting in approximately 50 mg /ml for injection.

Animals: Twenty Hubbard broiler chickens , weighing between 1.5 kg and 2 kg were used for the study . The birds were housed in cages, fed on antibacterial-free diet and had a free access to drinking water.

Artificial infection. *E. coli* (strain O_{76}), was kindly supplied by serology unit, Animal Health Research Institute. A concentration of 3X 10^6 C.F.U/ ml was used in the experiment (**Mossad *et al.,* 2015b**).

After inoculation with *E. coli* (strain O_{76} - $3x10^6$ CFU/ml) intraperitoneally, the clinical signs were observed before administration of clarithromycin and during the course of the experiment. The liver and spleen of the group 3 and 4 were excised aseptically. Samples from liver and spleen were cultured on peptone water at 37 °C for 24 h and plated on MacConkey agar. The identity of the *E. coli* strain was confirmed by agglutination test using O_{76} antiserum. **(Shen *et al.*, 2002)** .

Experimental design: Chickens were individually weighed before drug administration and doses were calculated precisely for each bird. Chickens were randomly divided into four groups (n = 5 each).

Group I : chickens received single IV bolus of 10 mg /kg b.wt of clarithromycin in the wing vein. (**Hanady *et al.,* 2016)**

Group 2: chickens were injected with 10 mg /kg b.wt of clarithromycin by IM route

(single dose). (**Hanady *et al.*, 2016).**
Group 3 and 4: were inoculated with *E. coli* (strain O_{76} - $3x10^6$CFU/ml) intraperitoneally **(Shen *et al.*, 2002)** and after appearance of symptom, the group 3 and 4wereinjected with10 mg /kg b.wt of clarithromycin by intramuscular and intravenous routes respectively.

Sample collection. Blood samples were collected from wing vein following intravenous or intramuscular administration in normal and experimentally infected chickens. Blood samples are collected after 0.083, 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours of administration. Serum was separated by centrifugation at 2000g for 10 minand stored at - 20 °C until assay **(Abo-El-Sooud *et al.* , 2012).**

Analytical procedure : The concentration of clarithromycin in serum samples was estimated by microbiological assay technique using *Bacillus subtilis* ATCC 6633 as tested organism. The test organism was cultured on nutrient agar at 37 ^{0}C for 24 h **(Arret *et al.*, 1971 and Tsai and Kondo, 2001**). Standard curve of serum clarithromycin was linear between 0.015 and 16 µg /mL. The concentrations of clarithromycin in serum was calculated from the standard curve.
Antibacterial activity in vitro (Minimum inhibitory concentration): Determination of minimum inhibitory concentration (MIC) by using Macro-broth dilution method for *E. coli, Klebsiella pneumoniae , Pseudomonas aeruginosa* according to **(Siddiqi *et al.*, 1993)**. Micro broth method was performed for *Mycoplasma gallisepticum* as described by **(Hannan, 2000).**

Pharmacokinetic analysis: The pharmacokinetic parameters were calculated by PK Solver: An add-in program for Microsoft Excel, version 2 **(Zhang *et al.*, 2010).** The data were expressed as (Mean ± SE) and analyzed using SPSS (16) software (SPSS Inc., Chicago, USA) .

The statistical analysis was performed using the Student′s t-test with $P<0.05$ as the level of significance **(Guan *et al.*, 2014).**

The time for which the serum drug levels remain above or equal to minimal inhibitory concentration (MIC) value is calculated using the formula **(Turnidge, 1998):**

$$\%T > MIC = In\left[\frac{D}{Vd(area) \times MIC}\right] \times \left[\frac{t1/2\beta}{In\,(2)}\right] \times \left[\frac{100}{DI}\right]$$

where :
T> MIC is the time interval (%) during which the serum concentration is above or equal to the MIC values.

In is natural logarithm
D is the proposed dose.
Vd (area) is the volume of distribution.
$\mathbf{t_{1/2\beta}}$ is the terminal elimination half-life.
DI is the dose interval .

Results
The chickens inoculated with E. coli (strain O_{76}) developed rough feathers and loss of appetite. The broiler chickens suffered from diarrhea by 24 h post- inoculation.
E. coli were re-isolated from liver and spleen. The treatment with clarithromycin was started when infected broiler chickens had developed significant clinical signs (after 24 h post- inoculation).
Clarithromycin serum concentration–time curves in healthy and *Escherichia coli*- infected broiler chickens following IV injection are shown in **(Figure 1) .**

In Escherichia coli- infected broiler chickens, the serum concentrations were lower than those registered in healthy ones.
Following IV injection, Pharmacokinetic parameters are presented in **(table 3).** As compared with healthy chickens, the values of A, a, B, $K_{12,21}$, $t_{0.5(\beta)}$,C^O, AUC, MRT were significantly higher in control chickens.
Clarithromycin serum concentration–time curves chickens following intramuscular dosing are illustrated in **(figure2)**. The serum con-

centrations in healthy group were also significantly higher than those in infected broiler chickens.

Pharmacokinetic parameters after intramuscular administration are presented in **(table 4)**.

After single IM administration, maximum serum concentration (C_{max}) , AUC , B , K_a, V/F,CL/F,V2/F were higher in healthy chickens. The absorption rate constant (k_{ab}) is significantly higher than the elimination rate constant (k_{el}).

Discussion

Pharmacokinetic parameters of clarithromycin was examined in this study after administration of 10 mg /kg b.wt of clarithromycin in healthy or E.coli infected broiler chickens .

Evaluation of results indicated that pharmacokinetics of clarithromycin after intravenous and intramuscular administrations in both healthy and infected chickens followed a two compartment open model. This conclusion is in agreement with that found in previous studies of clarithromycin carried out in human male volunteers **(Aftab *et al.*, 2001).**

Following a single intravenous injection of clarithromycin in healthy and infected chickens in a dose of 10 mg/kg , serum concentration of the drug is lower in infected chickens as compared to the healthy group after injection at the various time intervals. This could be attributed to a more rapid extra-vascular distribution of clarithromycin in diseased chickens than the healthy group. The phenomenon of rapid and wide distribution of the antimicrobial drugs in the diseased tissues has been previously reported in animals **(Baggot, 1980),** similar to that reported in *E. coli* infected chickens treated by enrofloxacin (**Soliman, 2000**), where the serum concentration was lower than in the healthy group.

The significant high value of the distribution rate constant (α) in healthy chickens **(6.54 ± 0.431/h)** After I/V administration indicates that clarithromycin is more rapidly distributed into various body fluids and tissue compartments than infected chickens **(5.89 ± 0.20 1/h)**. The rapid distribution of clarithromycin in control chickens is further substantiated by high values of K_{12}/K_{21} (3.78 ± 0.32 1/h , 2.51 ± 0.11 1/h, respectively). Similar results have been reported by **(Hanady *et al.*, 2016)** who found that clarithromycin was rapidly distributed with a short T½α (0.38 h), which is the time taken for the blood concentration of the drug to decline by 50% during the distribution phase of the disposition curve. Also the above results in agreement with that reported in *E. coli* lip polysaccharide induced febrile buffalo calves injected by cefepime **(Bharat and Suresh , 2009).**

AUC_{0-t} for clarithromycin in healthy chickens after IV and IM administration are (23.55 ± 0.15 µg/ml*h and 16.43 ± 0.65 µg/ml*h respectively), significantly higher in comparison to infected chickens. Data are different from those of (**Hanady *et al.*, 2016)** who found that AUC_{0-t} was 13.62 ± 1.230 µg/ml*h and 8.970 ± 0.707 µg/ml*h in broilers following admistration of 7.5mg/kg of clarithromycin by IV and intragatric routes respectively. This difference could be due to a difference in the dose and route of administration. The result also is in agreement with recorded by **(Dardi *et al.*, 2005)** who reported higher AUC0-t for ceftriaxone in healthy buffalo group than *E. coli* endotoxin induced fever group.

Following intramuscular administration ,the infection by *E. coli* significantly decreased the absorption rate of clarithromycin (Kab : 1.56 ± 0.09 1/h), C_{max} (1.66± 0.02 ug/ml) and AUC_{0-t} (13.53 ± 0.14 ug/ml*h), but increasedT_{max}(1.09 ± 0.03 h). This result was close to that determined for clarithromycin in foals by (**Jacks *et al.*, 2002)** where he found that the T_{max} was 1.5 hours and peak serum concentration (C_{max}) was 0.92 ug/ ml after intragastric administration of clarithromycin in foals at a dose of 10 mg/kg (bw). This difference could be due to differences in the dose

used or species. In a similar study, a low chloramphenicol plasma level was observed in *E. coli* infected veal calves **(Groothuis *et al.*, 1979).**

In *E.coli* infected group, a faster elimination of the drug was observed. A significantly lower volume of distribution (V/F : 3.26 ± 0.08 (mg) / (µg/ml)), elimination half-life ($t_{0.5(\beta)}$: 6.04 ± 0.20 h) and mean residence time (MRT : 8.4 ± 0.28 h), in addition to a higher elimination rate constant (b: 0.13 ± 0.004 1/h) and total body clearance (CL/F : 0.69 ± 0.01 (mg)/(µg/ml)/h) . This result is in accordance with (**Jacks *et al.*, 2002)** who found that the elimination rate constant and elimination half-lifeof clarithromycin was 0.12 ± 0.031/hand 4.81 h in foals and also was close to that reported with **Katayoun *et al.*, (2014)** who found that the elimination half-life of clarithromycin was about 3 to 4 hours with 2×250 mg tablet in Iranian Healthy Volunteers and also with that determined for clarithromycin in foals (5.4h) by **Womble *et al.*, (2006)**.

E. coli causes a high degree of inflammation and profound clinical abnormalities (e.g., fever, disseminated intravascular coagulation and hypotension); these factors may significantly influence hepatic and renal clearance or plasma protein binding of drugs, thereby affecting both metabolism and excretion **(Danuta *et al.*, 2016).**

(Etuk and Onyeyili, 2006) reported that fever and inflammation are cardinal features in bacterial infection, which may in turn causes an increase in heart rate and cardiac output, increasing blood flow to the liver and kidneys, all these could lead to increase in the rate at which the drug is delivered to both organs which are important sites of drug excretion**.** The above may somewhat explain the increase in the total body clearance in infected chicken.

In comparison, infected chicken showed no significantly difference in IM bioavailability (71.18±1.32%) than healthy chickens (69.76 ± 2.84%). This result is in agreement with the previous studies (**Hanady *et al.*, 2016).** The result indicates a good absorption of clarithromycin after IM administration.

Although the medical use of clarithromycin has not been extended to poultry, a few publications have reported the in-vitro antibacterial activities against bacteria of high incidence in poultry farms. The determined MIC value was 12.9 ± 5.60 µg/ml, 0.78 ± 0.05 ug/ml , 10.4 ± 3.61 ug/ml and 8.3±3.60 ug/ml against *Mycoplasma gallisepticum,. E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* respectively **(table 1).**

The design of a dosing regimen begins with an assessment of the minimal inhibitory concentration (MIC) of the antibacterial agent for a particular pathogen. Depending on the antimicrobial, serum or tissue drug concentrations should either markedly exceed the MIC by 10 to 12 fold for concentration or T> MIC should be at least 50% of the dosage interval to ensure an optimal bactericidal effect **(Toutain and Lees, 2004**).

According to above results , MIC against *Mycoplasma gallisepticum*, *Klebsiella pneumoniae, Pseudomonas aeruginosa* was above the maximum serum concentration of the drug (C_{max} = 2.07 ± 0.06 ug /ml). On the other side, MIC against *E. coli* (0.78 ±0.05 ug/ml) was below the serum concentration of the drug, so clarithromycin by the dose 10mg/kg b.wt. can treat *E. coli* infection and not prefer to use in other above bacterial infection .

The experimental data presented here show that clarithromycin at a dose of 10 mg/kg b.wt. at 24 h interval is sufficient to maintain T> MIC above 50% following IV in healthy and infected chickens (for healthy chicken = 69.8 ± 0.49% and the infected group= 57.57 ± 0.42%) and at 12 h interval following IM injection (healthy= 64.27 ± 0.67 % and infected =72.30 ± 0.82%) for *E. coli*. This dosage regimen meets pharmacokinetic–pharmacodynamic cri-

teria predicting a successful therapy for susceptible bacteria with MIC ≤ 0.78ug/mL.

These findings provide strong evidence that it is essential to optimize the usage and dosage of clarithromycin in *E. coli* infected broiler chickens for a better treatment of colibacillosis. Also, from the present study, we should be aware that a healthy animal model cannot substitute for an infection animal model to evaluate the pharmacokinetics of clarithromycin in broilers.

Conclusion

This study revealed that administration of a therapeutic dose of clarithromycin is effective in treating E. coli (strain O_{76}) infection in chickens. Pharmacokinetic parameters of clarithromycin were lower in infected chickens compared to normal ones. Intramuscular administration of clarithromycin 10 mg/kg at 12h interval appeared to be the suitable dosage regimen in infected chickens by *E. coli*.

Table (1). MIC (ug / ml) of clarithromycin against some different bacterial strains.

Type of bacteria	MIC (ug / ml)
Mycoplasma gallisepticum	12.9 ±5.60
E . coli	0.78 ±0.05
Klebsiella pneumoniae ,	10.4 ±3.61
Pseudomonas aeruginosa	8.3±3.60

Table (2). Calculated T> MIC for clarithromycin based on the estimated pharmacokinetic parameters obtained following IV and IM injection of 10 mg/kg body weight in healthy and *E.coli* infected broiler chickens (MIC =0.78 ±0.05 ug/ml) for 12 and 24 h dosing interval.

Parameters	T> MIC			
	24 h		12 h	
	IV	IM	IV	IM
healthy chickens	69.8 ± 0.49 %	32.29 ±0.57 %	138.94. ±.0.74 %	64.27 ± 0.67 %
Infected chickens	57.57 ± 0.42 %	36.32 ± 0.59%	114.58± 0.91 %	72.30± 0.82 %

Table (3), Pharmacokinetic parameters of clarithromycin following a single intravenous dose (10 mg/kg b.w) in healthy and *Escherichia coli*- infected broiler chickens . (Mean ± SD). (n = 5)

Parameter	Unit	Healthy chickens	*E. coli*- infected broiler chickens
A	µg/ml	5.87 ± 0.31	4.93 ± 0.16*
a	1/h	6.54 ± 0.43	5.89 ± 0.20*
B	µg/ml	3.46 ± 0.04	3.13 ± 0.05*
b	1/h	0.14 ± 0.01	0.16 ± 0.01
k_{el}	1/h	0.38 ± 0.01	0.41 ± 0.02
$t_{0.5\beta}$	h	4.66 ±0.09	4.11 ± 0.14*
k_{12}	1/h	3.78 ± 0.32	3.25 ± 0.12*
k_{21}	1/h	2.51 ± 0.11	2.39 ± 0.09*
$t_{0.5a}$	h	0.10 ± 0.01	0.11 ± 0.004
Cp^0	µg/ml	9.33 ± 0.34	8.05 ± 0.18*
V	(mg)/(µg/ml)	1.07 ± 0.04	1.24 ± 0.03
CL	(mg)/(µg/ml)/h	0.41 ± 0.004	0.51 ± 0.01
V_2	(mg)/(µg/ml)	1.60 ± 0.03	1.68 ± 0.04
CL_2	(mg)/(µg/ml)/h	4.04 ± 0.20	4.03 ± 0.08
AUC_{0-t}	µg/ml*h	23.55 ± 0.15	19.11± 0.40*
AUC_{0-inf}	µg/ml*h	24.21 ± 0.21	19.44 ± 0.45*
AUMC	µg/ml*h^2	157.16 ± 4.75	110.82 ± 6.35*
MRT	h	6.48 ± 0.14	5.69 ± 0.20*
$V_{d(ss)}$	mg/(µg/ml)	2.67 ± 0.03	2.92 ± 0.04

A - zero time intercept of the distribution phase, a: Distribution rate constant, B : zero time intercept of the elimination phase, b: Elimination rate constant, k_{el}: elimination rate constant,$t_{0.5\beta}$: elimination half-life; K_{12} and K_{21}: first-order rate constants for drug distribution between the central and peripheral compartments, $t_{1/2a}$:distribution half-life;Cp^0: serum drug concentration at t=0, V: The apparent volume of central compartment; Cl: total body clearance; V_2: The apparent volume of peropheral compartment; CL_2:inter-compartmental clearances; AUC0-t : area under the curve ; AUC_{0-inf} : area under the curve from zero to infinity; AUMC: is the area under the first moment curve; MRT: mean residence time, Vdss: volume of distribution at steady state ;

Table (4). Pharmacokinetic parameters of clarithromycin following a single intramuscular dose (10 mg/kg b.w.) in healthy and Escherichia coli- infected broiler chickens . (Mean ± SD). (n = 5)

Parameter	Unit	Healthy chickens	*E. coli*- infected broiler chickens
a	1/h	0.92 ± 0.22	1.40 ± 0.05
B	µg/ml	1.94 ± 0.34	1.63 ± 0.04*
b	1/h	0.11 ± 0.01	0.13 ± 0.004
$t_{0.5a}$	h	1.46 ± 1.18	0.49 ± 0.01
K_{ab}	1/h	2.37 ± 0.17	1.56 ± 0.09*
$t_{0.5ab}$	h	0.29 ± 0.02	0.44 ± 0.02
k_{el}	1/h	0.15 ± 0.01	0.21 ± 0.005
$t_{0.5(\beta)}$	h	6.11 ± 0.53	6.04 ± 0.20
k_{12}	1/h	0.22 ± 0.05	0.55 ± 0.021
k_{21}	1/h	0.79 ± 0.06	0.76 ± 0.05
V/F	(mg)/(µg/ml)	3.68 ± 0.23	3.26 ± 0.08*
CL/F	(mg)/(µg/ml)/h	0.57 ± 0.02	0.69 ± 0.01*
T_{max}	h	1.06 ± 0.08	1.09 ± 0.03
C_{max}	µg/ml	2.07 ± 0.06	1.66 ± 0.02*
AUC $_{0-t}$	µg/ml*h	16.43 ± 0.65	13.53 ± 0.14*
AUC $_{0-inf}$	µg/ml*h	17.47 ± 0.67	14.48 ± 0.22*
AUMC	µg/ml*h^2	115.23 ± 6.47	128.33 ± 5.62*
MRT	h	8.85 ± 0.26	8.4 ± 0.28
F	%	69.76 ±2.84	71.18± 1.32

a: Distribution rate constant, B : zero time intercept of the elimination phase , b: Elimination rate constant, $t_{1/2a}$:distribution half-life, kab: absorption rate constant, $t_{1/2ab}$:absorption half-life, k_{el}: elimination rate constant, $t_{0.5\beta}$: elimination half-life; K_{12} and K_{21} : first-order rate constants for drug distribution between the central and peripheral compartments ,V: The apparent volume of central compartment, Cl: total body clearance , T_{max} : the time point of maximum serum concentration;
C_{max}: the maximum serum concentration, AUC $_{0-t}$: area under the curve ; AUC $_{0-inf}$: area under the curve from zero to infinity ,AUMC : is the area under the first moment curve; MRT: mean residence time , F: bioavailability.

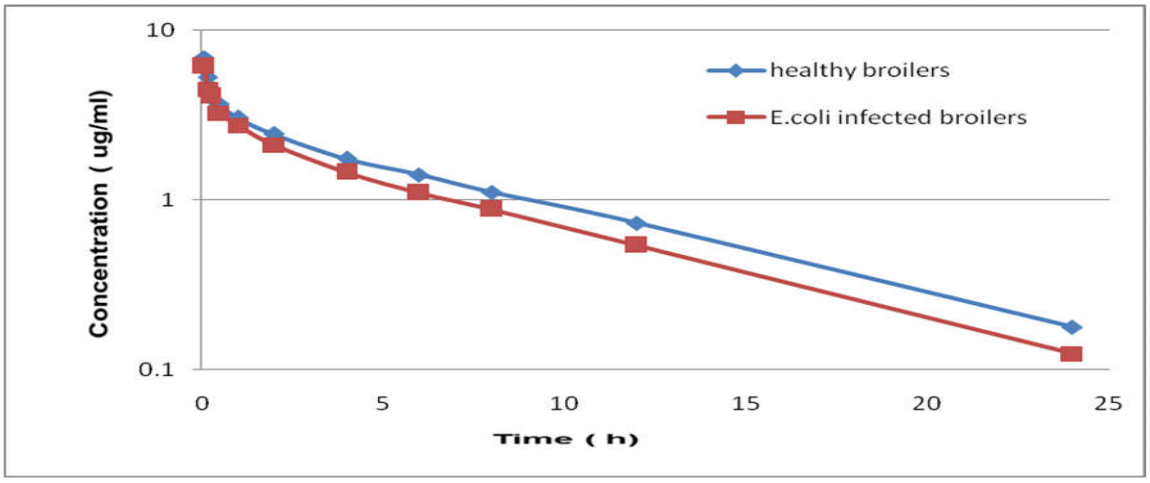

Figure (1). Semi logarithmic plot of comparative clarithromycin levels following a single intravenous administration (10 mg/kg B.W.) in healthy and Escherichia coli- infected broiler chickens . (Mean ± SD). Data presented as mean ± SD (P<0.05).

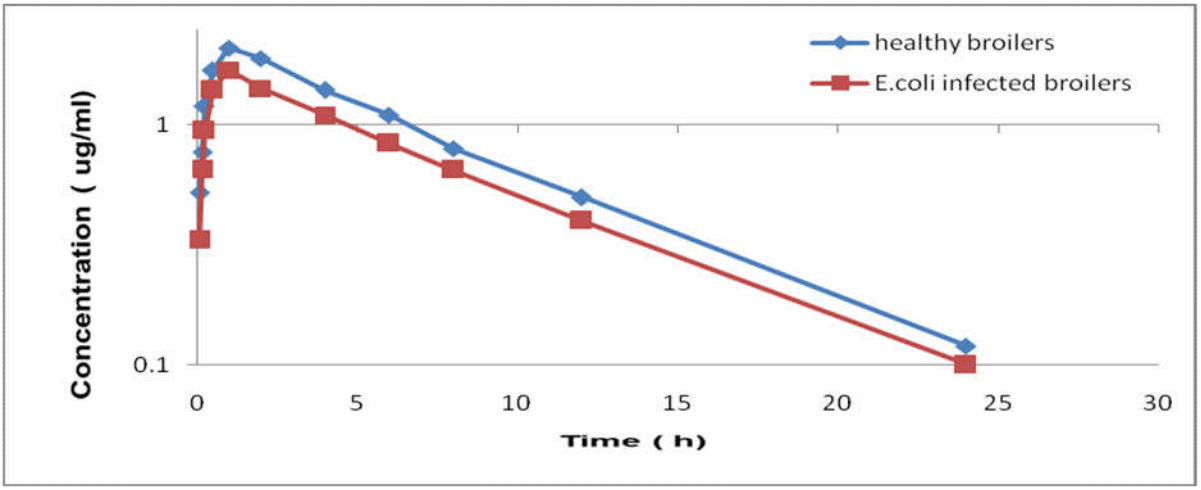

Figure (2). Semi logarithmic plot of comparative clarithromycin levels following a single intramuscular administration (10 mg/kg B.W.) in healthy and Escherichia coli- infected broiler chickens . (Mean ± SD). Data presented as mean ± SD (P<0.05).

References

Abo-El-Sooud, K.; Fahmy, E.; Afifi, N. and El-Aty, A.A. (2012). Pharmacokinetics and bioavailability of azithromycin following intramuscular and oral administrations in broiler chickens. Biosciences 6, 264-270.

Aftab, A.; Munir, A.S.; Muhammad, N.; Muhammad, S.; Abdul, Ghaffar and Shakeela, N. (2001). Biodisposition kinetics of clarithromycin following oral administration of 250mg tablet in human male volunteers. J. of Med. Sci., 1: 71 -73.

Alvarez-Elcoro, S. and Enzler, M.J. (1999). The macrolides: erythromycin, clarithromycin, and azithromycin. Mayo Clinic Proceedings,74, 613-634.

Arret, B.; Johnson, D.P. and Kirshbaum, A. (1971). Outline of details for microbiological assays of antibiotics: second revision," J. of Pharm. Sci., 60(11): 1689–1694.

Baggot, J.D. (1980). Distribution of antimicrobial agents in normal and diseased animals . J. of Am. Vet. Med. Associ. 176, 1085-1090.

Bharat, J. and Suresh, K.S. (2009). The pharmacokinetics of cefepime in E. coli induced febrile buffalo calves. Veterinarski Arhiv ,79, (6): 523-530.

Bryan, J.P.; Waters, C.; Seffield, J. and Wagner, K.F. (1990). In vitro activity of tosufloxacin (A-60969) and clarithromycin (A-56268, TE-031) against resistant Haemophilus influenza, Streptococcus pneumoniae and Branhamella catarrhalis isolates. Eur J. Clin. Microbiol. Infect. Dis., 9(5): 361-365.

Conte, J.E.; Golden, J.A.; Duncan, S.; McKenna, E. and Zurlinden, E. (1995). Intrapulmonary pharmacokinetics of clarithromycin and of erythromycin. Antimicrob. Agents and Chemother., 39, 334-338.

Danuta, S.F.; Hanna, B.; Tomasz, G.; Justyna, L.; Michał, M.; Hanna, U. and Edmund, G. (2016). Effects of Low-Dose Escherichia coli Lipopolysaccharide-Induced Endotoxemia on Morphine Pharmacokinetics in an Animal Model . Pain Medicine, Volume 17, Issue 8, 1, 1407–1415.

Dardi, M.S.; Sharma, S.K. and Srivastava, A.K. (2005). Pharmacokinetics and dosage regimen of ceftriaxone in E. coli lipopolysaccharide induced fever in buffalo calves. J. Vet. Sci. 6, 147-150.

Etuk, E.U. and Onyeyili, P.A. (2006). The effect of salmonella infection on the plasma kinetics of chloramphenicol in the Sokoto red goats. Inter. J. of Pharmacol., 2(1): 28-32.

Groothuis, D.G.; Gogh, H.V. and Van, M. (1979). The effect of Echrichia coli endotoxin induced fever on the blood levels of antimicrobial drugs after intravenous and intramuscular administration in veal calves. Trend in Vet. Pharmacol. Toxic. 6:77 86.

Guan, H.; Qian, D.; Ren, H.; Zhang, W.; Nie, H.; Shang, E. and Duan, J. (2014). Interactions of pharmacokinetic pro-

file of different parts from Ginkgo biloba extract in rats. J Ethnopharmacol., 8;155(1):758 -68.

Hanady, A.; Shaban, A. and Abubakr, E.M. (2016). Pharmacokinetics of clarithromycin after single intravenous and intracrop bolus administrations to broiler chickens .Inter. J. of Pharma. and Toxic., 4 (1): 12-18.

Hannan, P.C. (2000). Guidelines and recommendations for antimicrobial minimum inhibitory concentration (MIC) testing against veterinary mycoplasma species Vet. Res. 31 (4): 373-395.

Hardy, D.J.; Swanson, R.N.; Rode, R.A.; Marsh, K.; Shipkowitz, N.L. and Clement, J.J. (1990). Enhancement of the in vitro and in vivo activities of clarithromycin against Haemophilus influenzae by 14-hydroxy-clarithromycin, its major metabolite in humans. Antimicrob. Agents Chemother. 34 (7): 1407-13.

Hiroshi, N. (2009). Multidrug Resistance in Bacteria . Annu Rev Biochem. 78: 119–146.

Jacks, S.; Giguère, S.; Gronwall, R.R.; Brown, M.P. and Merritt, K.A. (2002). Disposition of oral clarithromycin in foals. J Vet Pharmacol. Ther.; 25(5): 359-62.

Katayoun, D.; Gholamreza, B.; Bahareh, M. and Elaheh, A. (2014). Oral bioavailability and pharmacokinetic study of clarithromycin in different dosage forms in iranian healthy volunteers. J. Bioequiv. 6 (6): 206-211.

Mossad, G.; Ashraf, A.; Elham, M. and Ahmed, M. (2015a). Pharmacokinetics and tissue residues of cefquinome in normal and salmonella entretidis infected chickens. World J. of Pharm. and Pharmaceut. Sci. vol 4, issue 10, 1974-1987.

Mossad, G.; Ashraf, A.; El barawy, A. and Ibrahim, D. (2015b). Pharmacokinetics and Tissue Residues of Ceftiofur in Normal and Escherichia Coli Infected Chickens. J. Phys. Pharm.Adv.,5(3): 574-582.

Patel, K.B.; Xuan, D.; Tessier, P.R.; Russomanno, J.H.; Quintiliani, R. and Nightingale, C.H. (1996). Comparison of bronchopulmonary pharmacokinetics of clarithromycin and azithromycin. Antimicrob. Agents and Chemother., 40, 2375-2379.

Shen, J.; Wu, X.; Hu, D. and Jiang, H. (2002). Pharmacokinetics of florfenicol in healthy and Escherichia coli-infected broiler chickens. Res .Vet. Sci. , 73(2):137-40.

Siddiqi, S.H.; Heifets, L.B.; Cynamon, M.H.; Hooper, N.M.; Laszlo, A.; Libonati, J.P.; Lindholm-Levy, P.J. and Pearson, N. (1993). Rapid broth macrodilution method for determination of MICs for Mycobacterium avium isolates. J Clin Microbiol. 31(9): 2332-2338.

Soliman, G.A. (2000). Tissue distribution and disposition kinetics of enrofloxacin in healthy and E. coli infected broilers. Deutsche Tieraztliche Wochencshrift, 107 :23 –27.

Steeve, G.; Stephanie, J.; Gregory, D.R.; Jorge, H.; Maureen, T.L. and Christina, E. (2004). Retrospective Comparison of Azithromycin, Clarithromycin, and Erythromycin for the Treatment of Foals with Rhodococcus equi Pneumonia J. Vet. Intern. Med., 18: 568–573.

Turnidge, J.D. (1998). The Pharmacdynamics of Beta-Lactams. Clic. Infect. Dis. 27, 10-22.

Toutain, P.L. and Lees, P. (2004). Integration and modeling of pharmacokinetic and pharmacodynamic data to optimize dosage regimens in veterinary medicine. J. Vet. Pharmacol. Ther. 27, 467-477.

Tsai, C. and Kondo, F. (2001). Improved agar diffusion method for detecting residual antimicrobial agents. J. Food Prot., 64: 361-366.

Womble, A.Y.; Giguère, S.; Lee, E.A. and Vickroy, T.W. (2006). Pharmacokinetics of clarithromycin and concentrations in body fluids and bronchoalveolar cells of foals. Am. J. Vet. Res. 67(10): 1681-1686.

Zhang, Y.; Huo, M.; Zhou, J. and Xie, S. (2010). PK Solver: An add in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft excel. Computer methods and programs in biomedicine; 99(3): 306.